Abstracts

Keynote Presentation

Reducing uncertainty in comparative risk assessments --- Research that makes a difference

Ray C. Loehr

H.M. Alharthy Centernnial Chair Emeritus,
The University of Texas at Austin
E-mail: r.loehr@mail.utexas.edu

Abstract

The objective of this Workshop is to identify possible research that might increase the use of bioavailability data in comparative risk assessments, particularly for soils and sediments containing petroleum hydrocarbons. The challenge is to identify specific topics, which if addressed by sound research, will reduce current uncertainties and thus make a difference in a risk assessment evaluation and in risk management decisions.

The intent of this presentation is to provide an overview of key points that could serve as a focus for discussions at the Workshop. Items that will be discussed include recognition of finite resources for research, the need for "use inspired R/D", the fact that different hydrocarbons have different site related and chemical characteristics, and the difference between chemical availability, environmental availability and environmental bioavailability.

Over the past decade, considerable research has occurred that relates to the topic of the Workshop. Many important items that have been learned in this period will be identified. Integrating the above topics will lead to suggestions of specific topics and a research strategy that appears appropriate, may stimulate further discussion and help lead to specific Workshop conclusions and recommendations.

Section 1

The role of soil organic matter structure in controlling the desorption and bioavailability of organic compounds

T.M. Young

Professor, Civil and Environmental Engineering Department,
University of California, Davis
E-mail: tyound@ucdavis.edu

Abstract

A portion of organic contaminants on soils and sediments is frequently found to resist desorption, extraction and biodegradation. Understanding of the mechanisms controlling these "sequestration" processes has been elusive. This talk reports on a series of studies examining how soil characteristics and aging time control the rate and extent of desorption and biodegradation of phenanthrene and on methods for assessing both soil structure and contaminant availability. Soil organic matter (SOM) structure has been assessed using quantitative pyrolysis GC/MS with external standards and solid state ^{13}C NMR. Amorphous and free iron and aluminum contents were also determined because these are hypothesized to be important loci of mineral-soil organic matter interactions, which may influence the configuration and accessibility of SOM domains within soil. Sorption/desorption isotherms were measured using phenanthrene as a probe compound for geosorbents with a wide range of organic carbon contents. Desorption rate measurements employed polymeric (Tenax) beads as an infinite sink. Inoculating sterilized soils with phenanthrene degrading bacteria was used to assess bioavailability. Correlations between 1) desorption and bioavailability and 2) the sorption and biodegradation properties and the soil characteristics were discussed in an effort to understand and predict biodegradation rates with desorption properties and soil characteristics as determinants.

Section 1

Does bioavailability limit biodegradation? A comparison of hydrocarbon biodegradation and desorption rates in aged soils

Michael H. Huesemann, *Richard Bartha, Tom S. Hausmann, and Tim J. Fortman*

Pacific Northwest National Laboratory,
Marine Sciences Laboratory,
1529 West Sequim Bay Road,
Sequim, WA 98382,
Phone: (360) 681-3618, Fax: (360) 681-3699, E-mail: michael.Huesemann@pnl.gov

Abstract

In order to determine whether bioavailability limits the biodegradability of petroleum hydrocarbons in aged soils, both the biodegradation and abiotic desorption rates of PAHs and n-alkanes were measured at various time points in six different aged soils undergoing slurry bioremediation treatment. Alkane biodegradation rates were always much greater than the respective desorption rates, indicating that these saturated hydrocarbons apparently do not need to be dissolved into the aqueous phase prior to metabolism by soil microorganisms. The biodegradation of PAHs was generally not mass-transfer rate limited during the initial phase, while it often became so at the end of the treatment period when biodegradation rates equaled abiotic desorption rates. However, in all cases where PAH biodegradation was not observed or PAH removal temporarily stalled, bioavailability limitations were not deemed responsible for this recalcitrance since these PAHs desorbed rapidly from the soil into the aqueous phase. Consequently, aged PAHs that are often thought to be recalcitrant due to bioavailability limitations may not be so and therefore may pose a greater risk to environmental receptors than previously thought.

Section 1

Activated Carbon Treatment of Contaminated Sediment to Reduce PCB Availability and Bio-uptake

Richard Luthy

Chair and Professor, Department of Civil and Environmental Engineering,
Stanford University, CA 94305
E-mail: luthy@Stanford.edu

Abstract

We propose that incorporating activated carbon (AC) into the biologically-active zone of contaminated sediment is an effective method to reduce the availability of persistent organic contaminants to the aqueous phase and biota. To test this concept, we contacted PCB-contaminated sediment from Hunters Point Naval Shipyard, San Francisco Bay, CA, with AC for one or six months and performed physicochemical and bioaccumulation tests with treated and untreated sediment. Sediment treated with 3.4% by weight AC showed 87% reduction in aqueous equilibrium PCB concentrations, and 78% reduction in the bioaccumulation of PCBs by the clam Macoma balthica, and 90% and 93% reduction in PCB uptake by Leptocheirus plumulosus and Neanthes arenaceodentata, respectively. Data from these and other tests suggest that the effectiveness of AC treatment depends on dose, contact time, and particle size. On-going tests at Hunters Point are evaluating field methods for mixing AC into sediment and the effectiveness in reducing PCB bioaccumulation by Macoma nasuta under field conditions.

Section 2

A biomimetic extraction approach for measuring bioavailability of PAH to Benthic Invertebrates

Donald Weston

Professor, Department of Integrative Biology
University of California, Berkeley, CA 94720
E-mail: dweston@berkeley.edu

Abstract

Standard chemical techniques typically extract far more PAH from soils or sediment than are biologically available. For benthic invertebrates, many of which are deposit feeders, the bioavailable fraction is constrained by the proportion of contaminant that is desorbed from the sediment during gut passage. We therefore have been investigating the potential of using deposit feeder gut fluids as a biologically relevant extractant in an in vitro procedure to quantify bioavailable PAH. Gut fluids typically extract 20-70% of PAH. Extraction efficiencies vary when using gut fluid from a variety of deposit feeders, but most taxa tested are fairly comparable with the exception of echinoderms, for which PAH extraction capability is similar to seawater. Comparisons between the fraction of contaminant solubilized in vitro and the fraction absorbed in vivo in actively feeding organisms have shown exceptionally good correlation, indicating the rapid in vitro extraction procedure is a reasonable surrogate for the more difficult in vivo measures of bioavailability. The major limitation to date of the gut fluid extraction procedure has been the limited availability of fluid because of the reliance upon deposit feeding invertebrates as a source of the extractant. However, it is clear that the effectiveness of gut fluid as a PAH extractant lies in the natural surfactants within the fluid, and recent results indicate a biomimetic solution containing a commercially available surfactant has PAH extraction capabilities comparable to gut fluid but without the supply limitations.

Section 2

Bioavailability Considerations in Aquatic Bioaccumulation of Hydrocarbons

Parkerton, T., D. Letkinski, E. Febbo, C. Dzamba, R. Blattenberger, M. Connelly

Toxicological and Environmental Sciences Division,
Exxon Mobil Biomedical Sciences, Inc.
Annandale, NJ 08801-0971
E-mail: Thomas.f.parkerton@exxonmobil.com

Abstract

Bioaccumulation is a useful measure of bioavailability. For aquatic organisms, such as fish, chemicals in respired water or ingested food can be taken across the gill and gut and accumulated in tissue. Once within the organism, compounds may exert an adverse biological effect in the fish and/or present a subsequent exposure source to human or wildlife consumers. In regulatory contexts, bioaccumulation is often quantified using bioaccumulation factors that are obtained using conservative models that rely on physical-chemical properties such as the octanol-water partition coefficient (K_{ow}). Since the hydrocarbon constituents that comprise petroleum substances typically exhibit high K_{ow} values, default bioaccumulation factors for these substances can be significantly exaggerated. To improve aquatic bioaccumulation assessment several important processes must be considered including: complexation of dissolved chemical to colloidal and particulate organic carbon; degradation in the gut; biotransformation in tissues and growth-dilution. To provide quantitative insights on the role these factors exert on the bioaccumulation of different hydrocarbon classes (n-paraffins, i-paraffins, olefins, naphthenes, aromatics) as a function of K_{ow}, a series of laboratory bioaccumulation experiments were performed. These tests involved spiking different hydrocarbons to a commercial diet. The spiked diet was then fed daily at a fixed ration to juvenile rainbow trout for a period of 1-2 weeks. The fish were then transferred to a clean tank and fed uncontaminated food. During this depuration period, fish were periodically sampled, weighed and analyzed for parent hydrocarbon using GC-MS. Based on these data, the growth-corrected half-life, dietary assimilation efficiency and biomagnification factor (BMF) for each hydrocarbon were determined. The BMF represents the steady-state, lipid-normalized concentration ratio of hydrocarbon in fish to diet and provides a diagnostic measure of biomagnification potential. The bioconcentration factor (BCF), which represents the steady-state concentration ratio in fish to that in water, was also derived from the experimentally derived half-life and the estimated uptake clearance that was calculated from the fish weight and a term accounting for the aqueous bioavailability of the hydrocarbon under test conditions. Based on test results, the dependence of BMF and BCF on K_{ow} for different hydrocarbon classes are presented and compared to default bioaccumulation model predictions. Study findings demonstrate the importance of (1) biotransformation in both gut and fish tissues and (2) aqueous bioavailability limitations in mitigating the bioaccumulation of hydrocarbons. These data also provide the technical basis for developing improved predictive bioaccumulation models for different hydrocarbon classes.

High molecular weight polycyclic aromatic hydrocarbon degradation by *Mycobacterium* species: Metabolism, proteomic and genomic approaches in the elucidation of PAH biodegradative pathways and implications in bioavailability

Carl E. Cerniglia

Director, Microbiology Division,
US Food and Drug Administration, National Center for Toxicological Research,
3900 NCTR Road, Jefferson, AR 72079
Phone: 870-543-7341, Fax: 870-543-7307, E-mail: ccerniglia@nctr.fda.gov

Abstract

Polycyclic aromatic hydrocarbons (PAHs) are introduced into the environment via many routes including fossil fuel combustion, manufactured gas and coal tar production, wood treatment processes, automobile exhaust, and waste incineration. Their presence in contaminated soils and sediments poses a significant risk to the environment since they have ecotoxic, mutagenic and in some cases carcinogenic effects. PAHs degrade relatively slowly through physical, chemical and biological processes, some of which are mediated by bacteria and fungi. The recalcitrance of PAHs increases with molecular weight, which is toxicologically important because many high molecular weight PAHs are genotoxic and carcinogenic. There long has been a question of whether bioremediation by bacteria and fungi can lower PAH levels enough to protect human health and ecological systems. Also monitoring the decrease in the PAHs that were initially present in a polluted site does not necessarily show the actual extent of detoxification because some PAH metabolites may themselves also be toxic. There are many bacterial and fungal species that can degrade PAHs including benzo[*a*]pyrene. PAHs are initially degraded by soil microorganisms by the dioxygenase, monooxygenase and peroxidase pathways, the first and second pathways are found in bacteria and the second and third in fungi.

Bacteria that oxidize PAHs include species of *Pseudomonas, Flavobacterium, Alcaligenes, Aeromonas, Vibrio, Sphingomonas, Bacillus, Nocardia, Pasteurella, Corynebacterium, Micrococcus, Mycobacterium, Acinetobacter, Arthrobacter, Streptomyces, Moraxella, Achromobacter, Rhodococcus, Comamonas, Microbacterium, Porphyrobacter, Thermus, and Burkholderia*. Most of the reported high molecular weight PAH degrading bacteria are Gram-positive including species of mycobacteria. They have been commonly isolated from PAH-contaminated soils. We found that soil mycobacteria have both PAH-hydroxylating dioxygenases and cytochrome P-450 monooxygenases which enhance their ability to degrade the highly recalcitrant PAHs.

Mycobacterium vanbaalenii PYR-1 was isolated from oil-contaminated sediment based on its ability to mineralize pyrene. This organism is capable of degrading high molecular weight polycyclic aromatic hydrocarbons (PAHs) including benz[*a*]anthracene and benzo[*a*]pyrene. The biodegradation pathways for anthracene, phenanthrene, fluoranthene, benzo[*a*]pyrene and benz[*a*]anthracene have been elucidated. The characterization of the major initial oxidation and ring-fission products indicated multiple routes of enzymatic attack in various positions on the aromatic ring. Protein expression profiles of *M. vanbaalenii* PYR-1 grown in the presence of

high molecular weight PAHs showed the over expression of at least sixteen proteins for the PAH treatment over uninduced control samples. Some proteins were found to be expressed uniquely upon exposure to specific PAHs, whereas others were common for more than one PAH.

Genomic approaches indicated that *M. vanbaalenii* PYR-1 uses multiple dioxygenases and cytochrome P-450 monooxygenases to degrade PAHs. PAH degrading genes that encode for the aromatic ring-hydroxylating dioxygenases have been cloned and sequenced from *M. vanbaalenii* PYR-1. In addition to the dioxygenases, three cytochrome P-450 genes were detected and the complete sequences determined. The genes for the lower pathway degradation enzymes have also been cloned and sequenced. The overall organization of the PAH degradation genes in *M. vanbaalenii* differs significantly from Gram-negative bacteria. *M. vanbaalenii* PYR-1 has been successfully used in field trials, bioreactor and microcosm studies to biotreat PAH contaminated sediments. These studies demonstrate the bioremediation potential of Mycobacterium species to degrade high molecular weight PAHs. In addition, these investigations provide fundamental information on PAH degradation as well as other metabolic processes in environmental strains of *Mycobacterium* spp.

Section 2

Assessing the bioavailability of PAHs to soil invertebrates: Theory, techniques, and applications

R.P. Lanno

Professor, Department of Entomology,
Ohio State University, Columbus, OH 43210
E-mail: lanno.1@osu.edu

Abstract

Soils are tremendously heterogeneous environmental matrices with varying spatial and temporal gradients of organic carbon, pH, and particle size distribution. These physical and chemical attributes of soil in turn determine the potential bioavailability of chemicals to soil-dwelling invertebrates. One of the biggest problems in determining the risk of hydrocarbons in soil to soil-dwelling ecological receptors is determining the actual chemical exposure of an organism. The expression of exposure and toxicity of chemicals in soils is often confounded by great differences between total chemical measures and the actual fraction of chemicals bioavailable for toxic action. Approaches for assessing the bioavailability of organic chemicals may be either biological or chemical in nature, but chemical measures must be correlated with biological responses. Bioaccumulation and critical body residues (CBRs) are a direct measure of bioavailability. Biomimetic sampling devices, such as solid-phase microextraction (SPME) techniques provide an indirect measure of chemical bioavailability that may correlate well with bioaccumulation. Chemical extraction techniques have also been used to assess the fraction of chemicals in soil that is bioavailable. This presentation will review chemical and biological approaches for assessing the bioavailability of chemicals for earthworms and discuss the application of these measures in the ecological risk assessment of PAHs.

Section 2

Controlled Measurements of the Uptake Pathways of Polycyclic Aromatic Hydrocarbons and n-alkanes from Soil into Wheatgrass

R.L. Maddalena and *T.E. McKone*

Environmental Chemistry, Exposure and Risk Group,
Environmental Energy Technologies Division,
Lawrence Berkeley National Laboratory, Berkeley, CA 94720
E-mail: rlmaddalena@lbl.gov

Abstract

Human and ecological risk assessments of contaminated soils often rely on bioconcentration factors (BCFs) that link soil residue levels to exposure concentrations in edible plant parts. When measured BCFs are lacking, which is most often the case, empirically-based models are used to estimate exposure concentrations in food or feed from soil residue levels. To date, these empirical relationships had to be based on a relatively small number of plant species and chemicals (mostly chlorinated hydrocarbons). As a result, linking chemical residues in soil to exposure concentrations is very uncertain. Few quantitative studies are available for reducing this uncertainty, particularly for petrogenic chemicals. To address this problem, we are using controlled environmental exposure chambers to measure the pathway specific uptake of twelve polycyclic aromatic hydrocarbons and six *n*-alkanes from spiked agricultural soils into wheat grass. Wheat is used as a model plant for this study because wheat grain and wheat containing products are widely consumed by both humans and farm animals. We reduce the levels of ambient pollutants in the exposure chamber air by passing the incoming air through a high efficiency filter and bed of activated carbon. We used a regression-based exposure design with multiple soil contaminant levels to differentiate between the direct soil-plant transfer and the indirect soil-air-plant uptake pathway. The measured logBCF values range from -1.0 to -2.9 for the twelve PAHs studied. The measured BCFs values are nonlinear with respect to logKow and are typically lower than those predicted from existing regression models. The presence of n-alkanes in the soil did not influence the magnitude of the BCFs for PAHs in grass.

Bioavailability assessment of organic pollutants in biaccumulation and toxicity studies using solid phase extractions

Joop LM Hermens

Institute for Risk Assessment Sciences,
Utrecht University,
PO Box 80176, 3508 TD Utrecht,
The Netherlands.
E-mail: j.hermens@iras.uu.nl

Abstract

Concentration measurements form the basis for the generation of numerous experimental parameters and many decisions are based on reported measured concentrations. Bioconcentration factors of highly hydrophobic compounds for example use aqueous concentrations in the denominator, although it is known that concentration measurements in aqueous solutions of hydrophobic chemicals are difficult to perform and can be subject to systematic errors. Total concentrations in soil and sediment are also often used by regulatory agencies in risk assessment decisions, while differences in bioavailability may strongly affect a site-specific risk. Also *in vitro* systems often report a dose or a concentration at a certain biological effect. However, as the precise exposure in these systems is often not investigated, quantitative data from these *in vitro* tests can be uncertain.

In this presentation, we focus on measured freely dissolved concentrations as a more intrinsic concentration parameter. We will show that effect concentrations based on freely dissolved concentrations represent the more intrinsic potency of chemicals in *in vitro* assays. Also examples will be presented on measured free concentrations of a few pesticides as well as PAHs in relation to bioavailability in soil.

A technique for measuring freely dissolved concentrations, based on solid phase micro-extraction, will be briefly discussed.

Acknowledgement:

I would like to acknowledge the contribution of Minne Heringa, Thomas ter Laak, Heather Leslie, and Leon van der Wal.

Predicting the bioavailability of PAHs from MGP soils and sediments using mild supercritical CO_2 extraction

Steve Hawthorne[a] and Joe Kreitinger[b]

[a]Energy and Environmental Research Center, University of North Dakota, Box 9018, Grand Forks, North Dakota 58202. E-mail: shawthorne@undeerc.org,
[b]The RETEC Group, Ithaca, New York. E-mail: jkreitinger@retec.com

Abstract

Extraction with supercritical carbon dioxide (SFE) is being developed as a rapid test to determine the bioavailability of PAHs from MGP soils and sediments. The SFE conditions were first developed on samples collected during a one-year bioremediation in a field plot. A 40 minute SFE test correctly predicted the degree of removal obtained for individual and total PAHs whether the removal was high (e.g., >80% for 2- and 3-ring PAHs), or low (e.g., <5% for 5- and 6-ring PAHs) [1]. Based on these results, 14 additional coal gas and oil gas soil samples were collected, and the "fast" fractions of 20 individual PAHs were determined using 120 minutes of SFE and compared to 120 days of water desorption. Good agreement was obtained between both methods regardless of whether the PAHs were found to be tightly bound, or readily available [2,3]. The same 14 samples were also used to determine earthworm toxicity and PAH uptake. SFE showed that the toxic samples had much higher "available" PAH concentrations than the non-toxic samples, even though the total PAH concentrations were not predictive of toxicity [4]. Removal of the "available" PAHs by SFE eliminated the toxicity, even though PAH concentrations remained high, demonstrating that SFE extracts biologically-relevant molecules [4]. For the non-toxic samples, the standard equilibrium partitioning model overpredicted the uptake of PAHs by one to three orders of magnitude. However, using the SFE "available" (rather than total) PAH concentrations and a measure of carbon quality was able to predict the concentrations of 2- to 6-ring PAHs in worm lipid within a factor of 10 [4].

Most recently, the toxicity of polycyclic aromatic hydrocarbons (PAHs) to the aquatic amphipod, *Hyalella azteca*, was measured in 43 sediment samples having a wide range in total (sum of 16) PAH concentrations (4 to 5700 mg/kg) collected from manufactured gas plant (MGP) sites. The survival and growth of *H. azteca* in 28-day bioassays was unrelated to total PAH concentration with 100% survival in one sediment having PAH concentrations of 1,730 mg/kg total PAHs, while no survival was observed in other sediment samples with concentrations as low as 54 mg/kg total PAHs. The EPA's equilibrium partitioning model predicted that 40 out of the 43 sediments should be toxic, but only 6 sediments actually caused mortality. However, the use of "available" PAH concentrations determined using SFE or by measuring the PAH concentrations in pore water correctly differentiated the 6 toxic sediments from the 36 non-toxic sediments. These results demonstrate that either pore water or SFE measurements of "available" PAHs is needed to accurately predict toxicity to *H. azteca.*

Reference:

1. Hawthorne and Grabanski, *Environ. Sci. Technol*. *34*, **2000**, 4103-4110.

2. Hawthorne, Poppendieck, Grabanski, and Loehr, *Environ. Sci. Technol. 35*, **2001**, 4577-4583.
3. Hawthorne, Poppendieck, Grabanski, and Loehr, *Environ. Sci. Technol. 36*, **2002**, 4795-4803.
4. Kreitinger and Hawthorne, manuscripts in preparation.

Assessing the dermal bioavailability of PAH from PAH-contaminated soils using in vitro percutaneous absorption techniques

Tim A. Roy,

Petrotec Inc.
Paulsboro, NJ 08066
E-mail: petrotec@prodigy.net

Abstract

Soils contaminated with coal or petroleum by-products may contain high concentrations of carcinogenic polynuclear aromatic hydrocarbons (PAH). An estimation of PAH dermal bioavailability from PAH-contaminated soils is critical in assessing human health risks associated with dermal exposure. *In vitro* percutaneous absorption using intact human skin is one way to estimate bioavailability in dermal exposures. Percutaneous absorption experiments with a variety of lipophilic materials and, in particular, PAH have demonstrated good correlation between *in vivo* and *in vitro* procedures for this class of compounds. Additionally, *in vitro* percutaneous absorption using human skin eliminates interspecies extrapolation and overestimates of dermal penetration (relative to humans) often observed with animal models. Unlike PAH in direct contact with skin, soil-sorbed PAH must first partition from the solid matrix to the outer layer of the skin (stratum corneum or SC) prior to penetration and diffusion. Published *in vitro* dermal penetration studies show that sorption on soil of individual PAH or PAH in petroleum or coal by-products significantly impedes PAH penetration through the skin relative to the neat material. The reduction is generally attributed to soil organic matter content and soil "weathering" or "aging" phenomena which may result in soil-bound chemicals becoming increasingly desorption resistant over time. These and several more recently completed *in vitro* dermal penetration experiments will be described which estimate the dermal penetration properties (flux rates) of PAH in (MGP tars and lampblack) contaminated soils based on the measured dermal flux of a surrogate PAH, benzo[a]pyrene (BaP). The experimentally measured BaP dermal flux values are used to estimate the dermal flux of target PAH and to further calculate the dermally absorbed dose (DAD), an essential component of the equation to determine the human cancer risk associated with dermal exposure to PAH.

What can *in vitro* digestion models add to human risk assessment of contaminated soil?

A.G. Oomen[1], C.R.M. Rompelberg[1], M. Duits[2], F.A. Swartjes[3], A.J.A.M. Sips[1].

[1] Centre for Substances and Integrated Risk Assessment, National Institute for Public Health & the Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands. E-mail: Adrienne.sips@rivm.nl

[2] Laboratory for Food and Residue Analysis, National Institute for Public Health & the Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands.

[3] Laboratory for Ecological Risk Assessment, National Institute for Public Health & the Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands.

Abstract

The *Remediation Urgency Method (SUS)*, the method in the Netherlands to determine the urgency of soil remediation, was introduced in 1994 in the framework of the Dutch Soil Protection Act. The methodology is based on human and ecological risk assessment and risks of contaminant migration (Swartjes, 1999). It is used by local governments to prioritise the remediation of contaminated sites in their district. Recently an inventory on shortcomings and needs for improvement of the current method among users has been made. Subsequently, options for improvement have been indicated (Lijzen et al. 2003). Regarding human risk assessment, research on the estimation of *relative* bioavailability in the human body for the soil ingestion exposure pathway (particularly for lead by children) was highly prioritised.

The *relative* bioavailability factor (relative F) is defined as a ratio of two bioavailability values. The present concepts for human exposure assessment (like in the Dutch CSOIL exposure model and probably all other European exposure models) set relative F to 1.0 for all contaminants. This implies that bioavailability of a contaminant ingested with soil is assumed to equalize the bioavailability of the same contaminant ingested with the matrix applied in the toxicity or epidemiological studies (e.g. tap water, food, olive oil, etc.), underlying the derivation of the reference dose (RfD). This concerns a conservative assumption, because in many cases *relative* F is expected to be smaller than 1. This will generally be caused by “the matrix effect”, i.e. bioaccessibility (F_B). We consider bioavailability as the resultant of four processes, i.e. 1) intake of a matrix + contaminant, 2) release of the contaminant from its matrix in the gastrointestinal tract (F_B = fraction which becomes bioaccessible), 3) transport across the intestinal wall into the Portal vein (F_A = fraction absorbed), and 4) passing through the liver without being metabolised (F_H = fraction passing the liver without being metabolised).

In order to quantify the effects of the matrix of ingestion on bioaccessibility we have developed a physiologically based *in vitro* digestion model (Oomen et al., 2003). The model results were compared with the results for the *in vivo* situation, together with five other European *in vitro* digestion models, assessing bioaccessibility of soil-bound lead in the human gastrointestinal tract under fasted and fed conditions (van de Wiele *et al*., submitted). These *in vitro* digestion models mimic the physiological conditions as occurring during the *in vivo* digestion of ingested soil, as it passes down the various compartments of the human gastro-intestinal tract, i.e. the mouth, stomach, and small intestine. The present and other *in vitro* digestion models have shown that in many cases only a fraction of the contaminant becomes bioaccessible (Oomen et al., 2003;

Oomen et al., 2002; Oomen et al., 2000; Ruby et al., 1999; Oomen et al., 2002), confirming the findings of in vivo studies that the matrix of ingestion can have a profound effect on actual exposure. These findings support the need for incorporation of a *relative* bioavailability factor in the risk assessment procedures.

Ideas on implementing information on bioaccessibility within the process of determining remediation urgency will be presented.

References

1. Lijzen J.P.A., Otte P.F., Kovar K., Swartjes F.A., Bloemen H., Hoogendoorn E., Krystek P., Ritsema R., Rompelberg C., Verschoor A. 2003. Progress report on the Evaluation of the Dutch method to determine urgency of soil remediation; analysis of restraints and exploration of possible solutions (In Dutch). *RIVM-report 711701033/2003* (*in Dutch*).
2. Swartjes, F. A.. 1999. Risk-Based Assessment of Soil and Groundwater Quality in the Netherlands: Standards and Remediation Urgency. *Risk Analysis*, **19 (6)**, 1235-1249.
3. Oomen A.G., Rompelberg C.J.M., Bruil M.A., Dobbe C.J.G., Pereboom D.P.K.H., Sips A.J.A.M. 2003 Development of an *in vitro* digestion model for estimating the bioaccessibility of soil contaminants, *Archives of Environmental Contamination and Toxicology* **44**, 281-287.
4. Van de Wiele T.R., Oomen A.G., Wragg J., Cave M., Minekus M., Hack A., Cornelis C., Rompelberg C.J.M., De Zwart L.L., Klinck B., Van Wijnen J., Verstraete W. and Sips A.J.A.M. Comparison of five in vitro digestion models with in vivo data: lead bioaccessibility in the human gastrointestinal tract. *Submitted.*
5. Oomen, A.G., Sips, A.J.A.M., Groten, J.P., Sijm, D.T.H.M., Tolls, J., 2000. Mobilization of PCBs and lindane from soil during in vitro digestion and their distribution among bile salt micelles and proteins of human digestive fluid and the soil. *Environ. Sci. Technol*. **34**, 297-303.
6. Oomen, A.G., Hack, A., Minekus, M., Zeijdner, E., Cornelis, C., Schoeters, G., Verstraete, W., Wiele, T.V.d., Wragg, J., Rompelberg, C.J.M., Sips, A.J.A.M., Wijnen, J.V., 2002. Comparison of five *in vitro* digestion models to study the bioaccessibility of soil contaminants. *Environ. Sci. Technol*. **36**, 3326-3334.
7. Ruby, M.V., Schoof, R., Brattin, W., Goldade, M., Post, G., Harnois, M., Mosby, D.E., Casteel, S.W., Berti, W., Carpenter, M., Edwards, D., Cragin, D., Chappell, W., 1999. Advances in evaluating the oral bioavailability of inorganics in soil for use in human health risk assessment. *Environ. Sci. Technol*. **33**, 3697-3705.

Section 3

In vitro and *in vivo* assessment of oral absorption of hydrocarbon residues

[1]*Hoi-Ying N. Holman*, [2]*Regine Goth-Goldstein*, [3]*Patricia Durbin*

[1]Department of Ecology, Earth Sciences Division, Lawrence Berkeley National Laboratory.
E-mail: hyholman@lbl.gov
[2]Environmental Chemistry, Exposure and Risk Group, Environmental Energy Technologies Division, Lawrence Berkeley National Laboratory. E-mail: R_Goth-Goldstein@lbl.gov
[3]Chemical Sciences Division, Lawrence Berkeley National Laboratory

Abstract

Soil ingestion through hand-to-mouth activities is a potential exposure pathway that is required to be evaluated to establish risk-based cleanup levels for hydrocarbon contaminated sites. In this presentation, we shall highlight our technology research and development effort which aims at improving the current understanding of factors controlling the bioaccessibility and the bioavailability of ingested hydrocarbon residues, and at helping site management to meet this regulatory requirement. Here hydrocarbon residues are defined as hydrophobic molecules that remain tightly-bound to soil particles after weathering, for example, petroleum hydrocarbon residues. Bioaccessibility is defined as the fraction of soil-bound hydrocarbon residues that is mobilized from soil particles, whereas bioavailability is the fraction of soil-bound hydrocarbons that enters the systemic circulation.

Although hydrocarbon residues are tightly-bound to soil particles, they can be mobilized from soil during digestion, and become available for intestinal absorption. The mobilization step, also called solubilization, occurs mostly in the upper small intestine due to the detergency characteristics of bile salts. The amount of hydrocarbon residues that are solubilized can be estimated *in vitro* by means of a physiologically-based small intestine (PSI) extraction model developed in our lab (*1*,*2*). *In vitro* solubilization and bioaccessibility experiments with total petroleum hydrocarbon (TPH) residues from different polluted sites have demonstrated that the solubility of TPH residues was significantly higher for soil contaminated with diesel than with crude oil; compared to the fasting state solubility, the solubility of TPH residues was much greater during fat digestion; the solubility of TPH residues was reduced by soil organic carbon (SOC).

The *in vitro* assessment of the absorption step has been a challenge. For a long time it was believed that all bioaccessible hydrocarbons would be absorbed through the intestinal wall into the portal vein and transported to the liver, a major site of presystemic metabolism and detoxification of xenobiotic compounds. However, results from our *in vitro* experiments (*3*) show that for polycyclic aromatic hydrocarbons (PAHs) considerable metabolism occurs in the intestinal wall because of induction of intestinal CYP1A1 and CYP1B1. Using the human Caco-2 cells in conjunction with the transwell system (an established model system to simulate human intestinal absorption of chemicals) to measure benz[a]pyrene (B[a]P) uptake, we found that a major fraction of BaP reaching the basolateral chamber (representing the portal blood) was metabolized, and that an even larger fraction of BaP metabolites were released into the apical chamber (representing the intestinal lumen). This metabolism is caused by the induction of various intestinal forms of P450 (including CYP1A1, CYP1B1 and other) by B[a]P and many

other PAHs, and has been reported, for example, in Caco-2 cells (*4,5*), rat small intestinal epithelial cells (*6,7*), and in mice (8). This pre-systemic metabolism can reduce the total amount of the parent PAHs that enter the systemic circulation and leads to a partial detoxification. If the metabolite(s) exerts toxicity, it should be considered in assessing risk, whereas, if innocuous, the metabolite(s) should not be considered.

To better understand the link between bioaccessibility and bioavailability of hydrocarbon residues, we have developed a mass-balance *in vivo* mouse model (9). *In vivo* experiments using female Swiss-Webster mice fed with PAH contaminated soil show that a significant fraction of PAHs was metabolized at the intestinal wall, and that the extent of metabolism was compound dependent with phenanthrene the most recalcitrant (*10*). We propose to use phenanthrene as a marker for "apparent bioavailability", which can be linked to bioaccessibility measurements from *in vitro* experiments.

Acknowledgement:

For their contributions to this technology research and development, we thank Dr. L. Lo of the Children Hospital at Oakland, Dr. S. Ebbe, Dr. Y. Mao, Mr. D. Aston, and Ms. K. Brook, J. Kengsoontra, B. Kullgren, M. Mei, M. Russell and I. Zubieta of the Lawrence Berkeley National laboratory. This work is supported in part by the National Energy Technology Laboratory (NETL) of the U.S. Department of Energy under Contract DE-AC03-76SF00098 at the Lawrence Berkeley National Laboratory.

References:

(1) Holman, H.-Y. N., **2000**. Gastrointestinal mimetic device. U.S. Patent No. 6,040,188.

(2) Holman, H.-Y. N., Goth-Goldstein, R., David Aston, Mao, Y., and J. Kengsoontra, **2002**. Evaluation of gastrointestinal solubilization of petroleum hydrocarbon residues in soil using an *in vitro* Physiologically-Based Model. *Environmental Science & Technology*, 36(6):1281-1286.

(3) Goth-Goldstein, R., Holman, H.-Y.N., and M. Russell, **1999**. *In vitro* model for intestinal uptake of benzo[a]pyrene. Environmental Energy Technology Division, LBNL-43752.

(4) Buesen, R., Mock, M., Nau, H., Seidel, A., Jacob, J., and A. Lampen, **2003**. Human intestinal Caco-2 cells display active transport of benzo[a]pyrene metabolites. *Chemico-Biological Interac.*, 142:201-221.

(5) Buesen, R., Mock, M., Nau, H., Seidel, A., Jacob, J., and A. Lampen, **2002**. Interaction between metabolism and transport of benzo[a]pyrene and its metabolites in enterocytes. *Toxicology and Applied Pharmacology*, 183: 168-178.

(6) Zhang, Q.-Y., Ding, X., and L.S. Kaminsky, **1997**. cDNA cloning, heterologous expression, and characterization of rat intestinal CYP2J4. *Archives of Biochemistry and Biophysics*, 340(2):270-278.

(7) Zhang, Q.-Y., Wikoff, J., Dunbar, D., Fasco, M., and L.S. Kaminsky, **1997**. Regulation of cytochrome p4501A1 expression in rat small intestine. *Drug Metabolism and Disposition*, 25(1):21-26.

(8) Brooks, R.A., Gooderham, J.J., Edwards, R.J., Boobis, A.R., and D.J. Winton, **1999**. The mutagenicity of benzo[a]pyrene in mouse small intestine. *Carcinogenesis*, 20(1):109-114.

(9) Holman, H.-Y. N., Durbin, P., Ebbe, S., and B. Kullgren, **2004**. Kinetics of absorption, tissue distribution and elimination of soil-bound hexadecane and benzo[a]pyrene following gavage administration in mice. Submitted.

(10) Holman, H.-Y. N., Durbin, P., Ebbe, S., Kullgren, B., Zubieta, I., Brooks, K., and M. Mei,, **2005**. Apparent bioavailability of lampblack-associated PAHs following gavage administration in mice. Submitted.

Section 3

Risk assessment of chemicals in drinking water and associated uncertainties

Anna M. Fan, Ph.D., DABT
Pesticide and Environmental Toxicology Section
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency
Oakland, CA
E-mail: afan@oehha.ca.gov

Abstract

Risk assessments of chemicals in drinking water are conducted to evaluate contamination situations, provide health guidance and support setting regulatory standards. Health protective concentrations are developed based on cancer (if appropriate) and non-cancer endpoints for establishing public health goals (PHGs). PHGs are levels of chemicals in water not anticipated to result in adverse health effects following long-term exposures. These are used as the health based considerations for Maximum Contaminant Levels, or MCLs. PHGs have been developed for about 70 chemicals, including benzene, benzo(a)pyrene, ethylbenzene, methyl tert-butyl ether, perchlorate, toluene and xylene. The risk assessments involve evaluation of human and animal data and the results are affected by the quality of available data. Uncertainties exist where there are data gaps. Some of the uncertainties are addressed by use of assumptions and defaults for inter- and intra-species extrapolation, sensitive populations, exposure duration, relative source contribution, pharmacokinetics, bioavailability, and database deficiency, among others. Research is needed to fill data gaps and reduce the uncertainties.

Section 4

Determining risk-based cleanup levels for Total Petroleum Hydrocarbons

Jonathan Leong and Matt Small

US EPA
San Francisco, CA 94105
Leong.jonathan@epa.gov

Abstract

Fuels are a mixture of hundreds of petroleum hydrocarbons. However, the human health and environmental risks associated with petroleum hydrocarbons are typically evaluated based only upon the concentrations of a limited number of chemicals of concern (COCs). These COCs are typically selected because of their toxicity, as in the case of benzene, toluene, ethyl benzene, xylene (BTEX), and benzo(a)pyrene(BaP); their potential for impacting water taste and odor, as in the case of methyl tert-butyl ether (MTBE); and a desire to reduce analytical and investigation costs by limiting the number of chemicals of concern. As a result, remediation has been terminated and cleanup approved at many petroleum contaminated sites where undetermined concentrations of unknown petroleum hydrocarbons may still remain in soil and ground water. These remaining petroleum hydrocarbons present a potential, but undefined, risk to human health and the environment.

Section 4

Potential Impacts of Hydrocarbon Availability on Risk-Based Criteria: The Lampblack Experience

Hans Stroo
RETEC Group
Ashland, OR 97520
E-mail: hstroo@retec.com

Abstract

Lampblack, the principal residue resulting from the pyrolysis of oil to produce gas, may contain high levels of polynuclear aromatic hydrocarbons (PAHs). Risk assessments for oil-gas manufactured gas plant (MGP) sites are primarily driven by the risks to human health posed by the carcinogenic PAHs, particularly benzo(a)pyrene (BaP). Yet lampblack binds these PAHs tightly, greatly reducing the availability of the contaminants for leaching or biological uptake. A multi-investigator study of the chemical and biological availability of PAHs in seven lampblack samples has resulted in a protocol for modifying risk assessments by incorporating availability data. The results indicate that the uptake via both oral ingestion and dermal contact is far lower than current default assumptions suggest. Dermal and ingestion absorption factors (DAFs and IAFs) were experimentally derived using published in vitro test protocols. Using these absorption factors in the standard California risk assessment equations increased the risk-based cleanup levels by a factor of 72 on average, with a range from 23 to 142 times the default level. The chemical measures of availability included an aqueous rate of release test and a supercritical fluid extraction (SFE) assay, which have been previously shown to be closely correlated to each other. The rapidly-released fraction, as determined by the SFE test, was closely correlated (r2=0.96) to the cleanup levels calculated for each sample using the site-specific DAFs and IAFs. These results suggest a tiered protocol for site-specific assessments, using a rapid and inexpensive screening-level chemical availability test as part of an initial tier of evaluation, and a combination of in vitro assays for higher-tiered, more intensive assessments.